Original Article

# CCDC68 predicts poor prognosis in patients with colorectal cancer: a study based on TCGA data

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**Background:** The prognostic value of coiled-coil domain containing 68 (CCDC68) in colorectal cancer (CRC) is unclear. We evaluated the role of CCDC68 in CRC based on The Cancer Genome Atlas (TCGA) database.

**Methods:** Patients with CRC were collected from TCGA. We determined CCDC68 expression using the Wilcoxon rank sum test. Logistic analysis was applied to study the relationship between CCDC68 expression and clinicopathologic features. Cox regression and the Kaplan-Meier method were used to determine the predictive value of CCDC68 on clinical outcomes in CRC patients. Gene Set Enrichment Analysis (GSEA) and the single-sample Gene Set Enrichment Analysis (ssGSEA) were also conducted to annotate the biological function of CCDC68.

**Results:** Reduced CCDC68 expression in CRC was significantly correlated with N stage [odds ratio (OR) =0.95 for N1/N2 *vs.* N0], M stage (OR =0.91 for M1 *vs.* M0), pathologic stage (OR =0.95 for stage III/stage IV *vs.* stage I/stage II), neoplasm type (OR =0.92 for rectum adenocarcinoma *vs.* colon adenocarcinoma), tumor protein 53 (TP53) status [OR =0.93 for Mut (mutant) *vs.* WT (wild type)], and kirsten rat sarcoma viral oncogene (KRAS) status (OR =0.97 for Mut *vs.* WT) (all P values <0.05). Kaplan-Meier survival analysis showed that low CCDC68 expression had a poorer overall survival (OS) (P=0.008), progression-free interval (PFI) (P=0.006), and disease-specific survival (DSS) (P=0.023). Cox regression analysis revealed that CCDC68 was a risk factor for OS (P=0.047), PFI (P=0.048), and DSS (P=0.038). GSEA demonstrated that the chemokine signaling pathway, the Janus kinase-signal transducers and activators of transcription (JAK-STAT) signaling pathway, high-affinity IgE receptor (FcεRI)-mediated nuclear factor-κB (NF-κB) activation, cell adhesion molecules (CAMs), complement cascade, FcεRI-mediated mitogen-activated protein kinase (MAPK) activation, intestinal immune network for immunoglobulin A (IgA) production, and Toll-like receptor signaling pathway were differentially enriched in the high CCDC68 expression phenotype, while the Wnt signaling pathway was significantly enriched in the low CCDC68 expression phenotype. SsGSEA found that CCDC68 expression was positively correlated with T helper 2 (Th2) and T helper cells.

**Conclusions:** CCDC68 expression may be a potential prognostic molecular marker for poor survival in CRC. Moreover, CCDC68 may participate in the development of CRC via multiple signaling pathways.

**Keywords:** Coiled-coil domain containing 68 (CCDC68); colorectal cancer (CRC); prognosis; The Cancer Genome Atlas (TCGA)

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## Introduction

Colorectal cancer (CRC) ranks third in incidence (10%) and second in mortality (9.4%), with an estimated 19.3 million new cases and nearly 10 million deaths in 2020, accounting for approximately 10% of cancer cases and deaths (1). Until 2035, the number of deaths from colon and rectal cancer in all countries is expected to increase by 60.0% and 71.5%, respectively (2). Worryingly, the statistics are shown an increase in the incidence and mortality of CRC in adults younger than 50 years, who are typically below the screening age (3-5). As the disease only becomes symptomatic at an advanced stage, early screening programs are expected to reduce morbidity and mortality from CRC. In view of this, the American Cancer Society has updated its screening guidelines, reducing the age of CRC screening from 50 years to 45 years for average-risk individuals (5). Considering the limitations of CRC screening methods, such as invasiveness, high cost, as well as low specificity and sensitivity, identifying new molecular biomarkers with predictive or prognostic significance in CRC has become an important issue.

The main prognostic marker in clinical application is carcinoembryonic antigen (CEA), which is used for tumor diagnosis and post-treatment monitoring, but the elevated CEA lacks specificity (6). BRAF and KRAS mutations have been shown to have prognostic significance in stage II and III microsatellite instable colon cancers (7), however, different opinions remain (8). Thus, identification of new biomarkers associated with tumor stage and prognosis is crucial to facilitate early diagnosis, prognosis assessment and treatment of CRC.

Coiled-coil domain containing 68 (CCDC68), also known as cutaneous T-cell lymphoma (CTCL) tumor antigen se57-1, is a 335 amino acid protein expressed in CTCL, bone marrow, colon, small intestine, spleen, testis, and trachea tissues. Se57-1 contains 1 coiled coil domain and is encoded by the *CCDC68* gene mapping to human chromosome 18. CCDC68 has been affirmed as a putative tumor antigen in 21% of CTCL patients (9), 17% of renal cell cancer patients (10), and 15% of CRC patients (11). Recent studies have reported that the expression of CCDC68 has a negative effect on the tumor biology of pancreatic ductal adenocarcinoma and is associated with well differentiated tumors (12). Increased CCDC68 expression has been shown to promote non-small cell lung cancer cell proliferation *in vitro* (13). The high expression of CCDC68 predicts poor prognosis in endometrial carcinoma patients (14). Moreover, CCDC68 is downregulated in 89% of patients with primary CRC, and its expression is highly correlated with the related gene copy number (15). However, the potential role and underlying mechanism of CCDC68 in CRC is not clear yet , and there are few reports on the relationship between CCDC68 and CRC. CCDC68 may be a novel candidate tumor suppressor gene (TSG) in CRC, but this hypothesis requires further biological verification.

Therefore, we aimed to prove the correlation between CCDC68 and CRC, and analyze the prognostic role of CCDC68 in CRC based on The Cancer Genome Atlas (TCGA). To this end, we analyzed the expression difference of CCDC68 in CRC and normal tissues based on the RNA sequencing (RNA-seq) data of colorectal tumors in TCGA. Subsequently, the correlation between CCDC68 expression and clinicopathological variables and prognosis was analyzed. Moreover, gene set enrichment analysis (GSEA) was used to reveal the functional pathways associated with CCDC68 and CRC. By analyzing the correlation between CCDC68 expression and immune infiltration, the possible mechanism of CCDC68 participation in CRC was explored. Thus, our results could potentially reveal new targets and strategies for the diagnosis and treatment of CRC. We present the following article in accordance with the REMARK reporting checklist (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-148/rc).

## Methods

### *RNA-sequencing data and bioinformatics analysis*

The gene expression profile data with clinical information from colon adenocarcinoma (COAD) (461 cases, Workflow Type: HTSeq (High-throughput sequence)-FPKM (The Fragments per Kilobase of transcript per Million mapped reads)) and rectum adenocarcinoma (READ) (172 cases, Workflow Type: HTSeq-FPKM) projects were collected

 

from TCGA (update to 2021.10. https://portal.gdc.cancer.gov/). Excluding RNA-seq data without clinical information, a total of 619 cases were obtained. Next, level 3 HTSeq-FPKM data were transformed into TPM (transcripts per million reads) for further analysis. Unavailable or unknown clinical features in 619 COADREAD patients were considered as missing values. This study was conducted in accordance with the Declaration of Helsinki (revised in 2013) and complied with the publication guidelines provided by TCGA, and does not include any research on human participants or animals by any author.

### *GSEA*

GSEA is an analytical method used to interpret gene expression data, which works by focusing on gene sets; that is, genomes with common biological functions, chromosomal location, or regulation (16). In this study, GSEA, performed by R package clusterProfiler (17), was used to elucidate the significant function and pathway difference between high- and low-CCDC68 groups. Gene set permutations were performed 1000 times for each analysis. The expression level of CCDC68 was used as a phenotype label. The pathways enrichment was analyzed based on the P.adjust (P.adj) (<0.05), false discovery rate (FDR) (<0.25), and normalized enrichment score (NES) (|NES| >1).

### *Immune infiltration analysis by single-sample Gene Set Enrichment Analysis (ssGSEA)*

The ssGSEA method was used to analyze the immune infiltration of CRC for 24 types of immune cells in tumor samples. According to the marker genes of 24 kinds of immune cells in the literature (18), the relative enrichment scores of each immune cell were quantified from the gene expression profile of each tumor sample. Spearman correlation and Wilcoxon rank sum test were used to explore the correlation between CCDC68 expression and the infiltration levels of immune cells, as well as the relationship between immune cell infiltration and the high- and low-CCDC68 expression groups.

### *Statistical analysis*

All statistical analyses were performed using R software (R version 3.6.3, https://cran.r-project.org/bin/windows/base/old/3.6.3/). The Wilcoxon rank sum test was used to compare the expression of CCDC68 in tumor and normal tissues, and in normal samples of Genome Tissue Expression (GTEx) combined with TCGA and tumor samples corresponding to TCGA. The Wilcoxon signed rank sum test was performed to compare the tumor tissues and paired adjacent normal tissues. The relationship between clinicopathologic features and CCDC68 expression was analyzed using the Wilcoxon rank sum test or Kruskal-Wallis rank sum test and logistic regression. Cox regression and the Kaplan-Meier method were used to analyze the clinicopathologic characteristics associated with overall survival (OS), progression-free interval (PFI) and disease-specific survival (DSS), deriving all data from TCGA pan-cancer clinical data resource (TCGA-CDR) (19). Multivariate Cox analysis was used to compare the influence of CCDC68 expression on survival along with other clinical characteristics. In Cox regression analysis, variables with P<0.1 in univariate Cox regression were incorporated into multivariate Cox regression. The cut-off value of CCDC68 expression was determined by its median value. P values were 2-sided and a P value <0.05 was considered to be statistically significantly different.

## Results

### *Differences in CCDC68 expression between tumor and normal tissues*

To reveal the differences in CCDC68 expression between tumor and normal tissues, 619 COADREAD tumor tissues and 51 normal tissues from TCGA were analyzed. We found that the expression of CCDC68 was dramatically lower in tumor tissues than in normal tissues (P<0.001, *Figure 1A*). At the same time, we also analyzed the expression of CCDC68 in 50 cases of cancer tissues and paired adjacent normal tissues. The results also showed that the expression of CCDC68 was lower in the tumor tissues (P<0.001, *Figure 1B*), indicating that CCDC68 may prevent colorectal carcinogenesis. Furthermore, a receiver operating characteristic (ROC) curve was used to analyze the efficiency of CCDC68 in distinguishing tumors from non-tumor tissues. The area under the curve (AUC) of CCDC68 was 0.959, suggesting that CCDC68 expression has a good ability to distinguish tumors from non-tumor tissues (*Figure 1C*).

In addition, we downloaded RNA-seq data in TPM format of TCGA and GTEx uniformly processed by the Toil process (20) from the University of California,

 

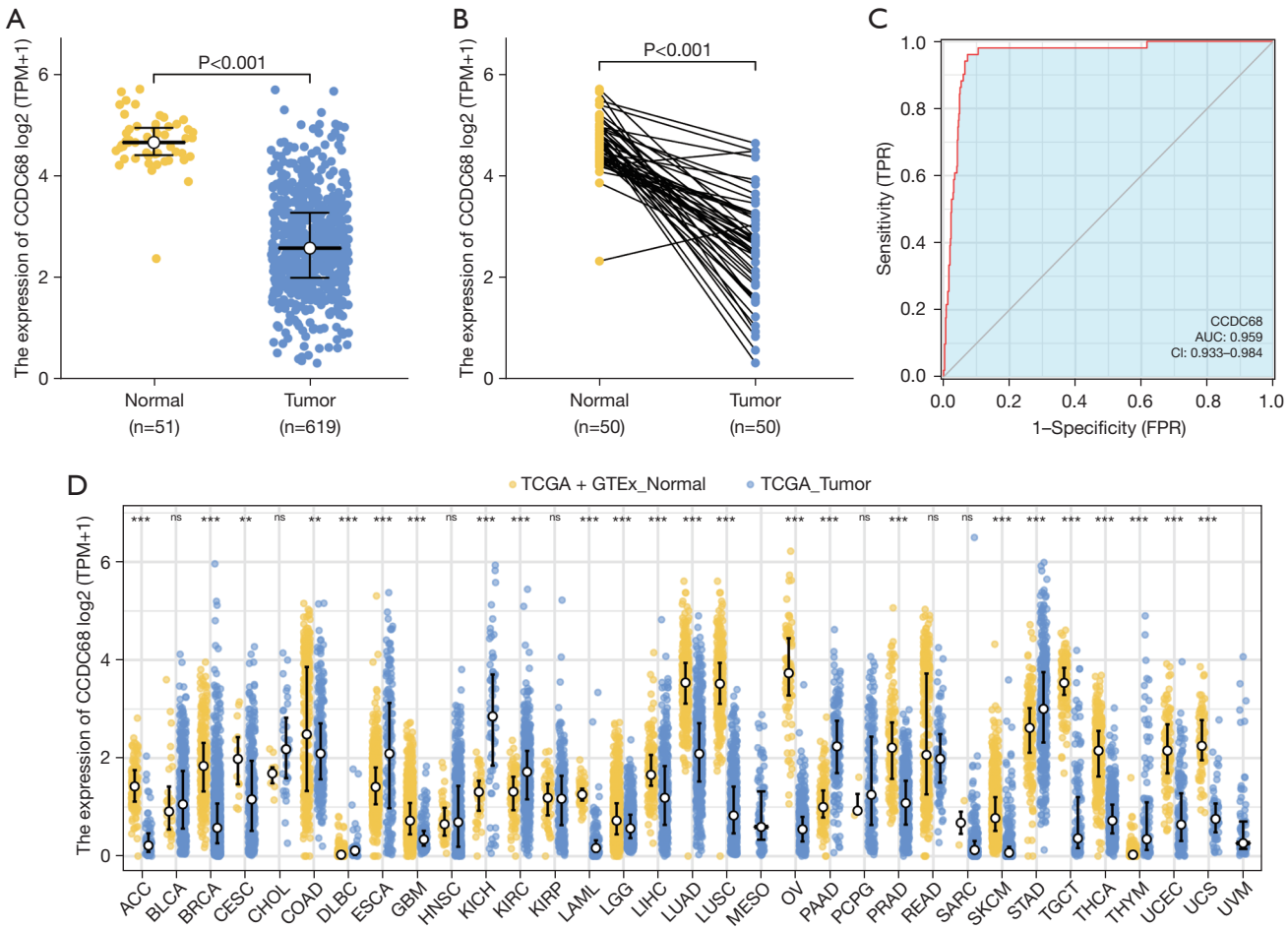


**Figure 1** CCDC68 expression in patients with CRC. (A) Expression differences in CCDC68 between tumor patients and normal samples, Wilcoxon rank sum test. (B) Expression differences in CCDC68 between tumor patients and paired adjacent normal samples, Wilcoxon signed rank sum test. (C) ROC curve showing the efficiency of CCDC68 in distinguishing tumors from non-tumor tissues. (D) Expression difference of CCDC68 between cancer tissues and control samples in 33 cancer types, Wilcoxon rank sum test. **, P<0.01; ***, P<0.001. ns, no significance, P≥0.05. CCDC68, coiled-coil domain containing 68; CRC, colorectal cancer; ROC, receiver operating characteristic; FPR, false positive rate; TPR, true positive rate; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma mutiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

Santa Cruz Xena (UCSC Xena) (https://xenabrowser.net/datapages/). As shown in *Figure 1D*, we compared the expression of CCDC68 in normal samples of GTEx combined with TCGA and tumor samples corresponding to TCGA with 33 cancer types using the Wilcoxon rank sum test. The results showed that CCDC68 expression was

 

significantly different in adrenocortical carcinoma (ACC), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), acute myeloid leukemia (LAML), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), testicular germ cell tumors (TGCT), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma (UCS), and the results were statistically significant (P<0.05).

### *Association between CCDC68 expression and clinicopathologic characteristics in CRC*

The Kruskal-Wallis rank sum test or Wilcoxon rank sum test were used to analyze the relationship between CCDC68 expression and clinicopathologic characteristics. Lower expression of CCDC68 was significantly associated with higher N stage (P<0.001), M stage (P<0.001), pathologic stage (P<0.001), residual tumor (P=0.007), neoplasm type (P<0.001), and TP53 status (P<0.001) (*Figure 2A-2F*). The association between the clinicopathologic characteristics of COADREAD in TCGA and CCDC68 high/low expression is shown in *Table 1*. The Chi-square test or Fisher's exact test showed that CCDC68 was significantly correlated with N stage (P<0.001), M stage (P<0.001), pathologic stage (P<0.001), neoplasm type (P=0.001), residual tumor (P=0.001), TP53 status (P=0.001), and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) status (P=0.045). Logistic regression was used to analyze the relationship between clinicopathologic features and CCDC68 TPM value of CRC. Reduced CCDC68 expression in CRC was significantly correlated with N stage (OR =0.95 for N1/N2 *vs.* N0, P=0.001), M stage (OR =0.91 for M1 *vs.* M0, P=0.003), pathologic stage (OR =0.95 for stage III/stage IV *vs.* stage I/stage II, P=0.002), neoplasm type (OR =0.92 for rectum adenocarcinoma *vs.* colon adenocarcinoma, P<0.001), TP53 status (OR =0.93 for Mut *vs.* WT, P<0.001), and KRAS status (OR =0.97 for Mut *vs.* WT, P=0.048) (*Table 2*). These results indicated that CRCs with low CCDC68 expression were prone to progress to a more advanced stage, as well as have lymph node and distant metastases compared to those with high CCDC68 expression.

### *Role of CCDC68 expression in the survival of CRC patients*

The Kaplan-Meier survival plot drawn by survminer R package (https://cran.r-project.org/package=survminer) was used to evaluate the prognostic value of CCDC68 in CRC. OS was significantly poorer in patients with low CCDC68 expression than those with high CCDC68 expression (HR: 0.62, 95% CI: 0.43–0.88, P=0.008), and a similar result was observed in PFI (HR: 0.64, 95% CI: 0.47–0.88, P=0.006) and DSS (HR: 0.59, 95% CI: 0.37–0.93, P=0.023) (*Figure 2G-2I*). Next, we performed univariate and multivariate analyses of the prognostic factors for OS, PFI and DSS using the Cox regression model (*Table 3*). In the univariate analysis, T stage (P=0.006), N stage (P<0.001), M stage (P<0.001), pathologic stage (P<0.001), CEA level (P<0.001), age P<0.001), residual tumor (P<0.001), and CCDC68 expression (P=0.008) were associated with OS, also T stage (P<0.001), N stage (P<0.001), M stage (P<0.001), pathologic stage (P<0.001), CEA level (P<0.001), race (P=0.036), residual tumor (P<0.001), KRAS status (P=0.033), and CCDC68 expression (P=0.006) were associated with PFI, whereas T stage (P=0.002), N stage (P<0.001), M stage (P<0.001), pathologic stage (P<0.001), CEA level (P<0.001), race (P=0.037), residual tumor (P<0.001), and CCDC68 expression (P=0.023) were associated with DSS. In the multivariate analysis, pathologic stage (P=0.020) and CCDC68 expression (P=0.048) were independent prognostic factors in OS (P<0.05), also M stage (P<0.001), CEA level (P=0.05), residual tumor (P<0.001), and CCDC68 expression (P=0.048) were independent prognostic factors in PFI (P<0.05), whereas N stage (P=0.022), M stage (P<0.001), residual tumor (P<0.001), and CCDC68 expression (P=0.038) were independent prognostic factor in DSS (P<0.05). The above data indicated that CCDC68 was a prognostic factor and decreased CCDC68 level was associated with poor OS, PFI and DSS.

### *CCDC68-related signaling pathways based on GSEA*

To identify the potential function of CCDC68, we performed GSEA of the low- and high-CCDC68 expression groups based on TCGA COADREAD expression matrix. In the Molecular Signature Database (MsigDB) Collections,

 

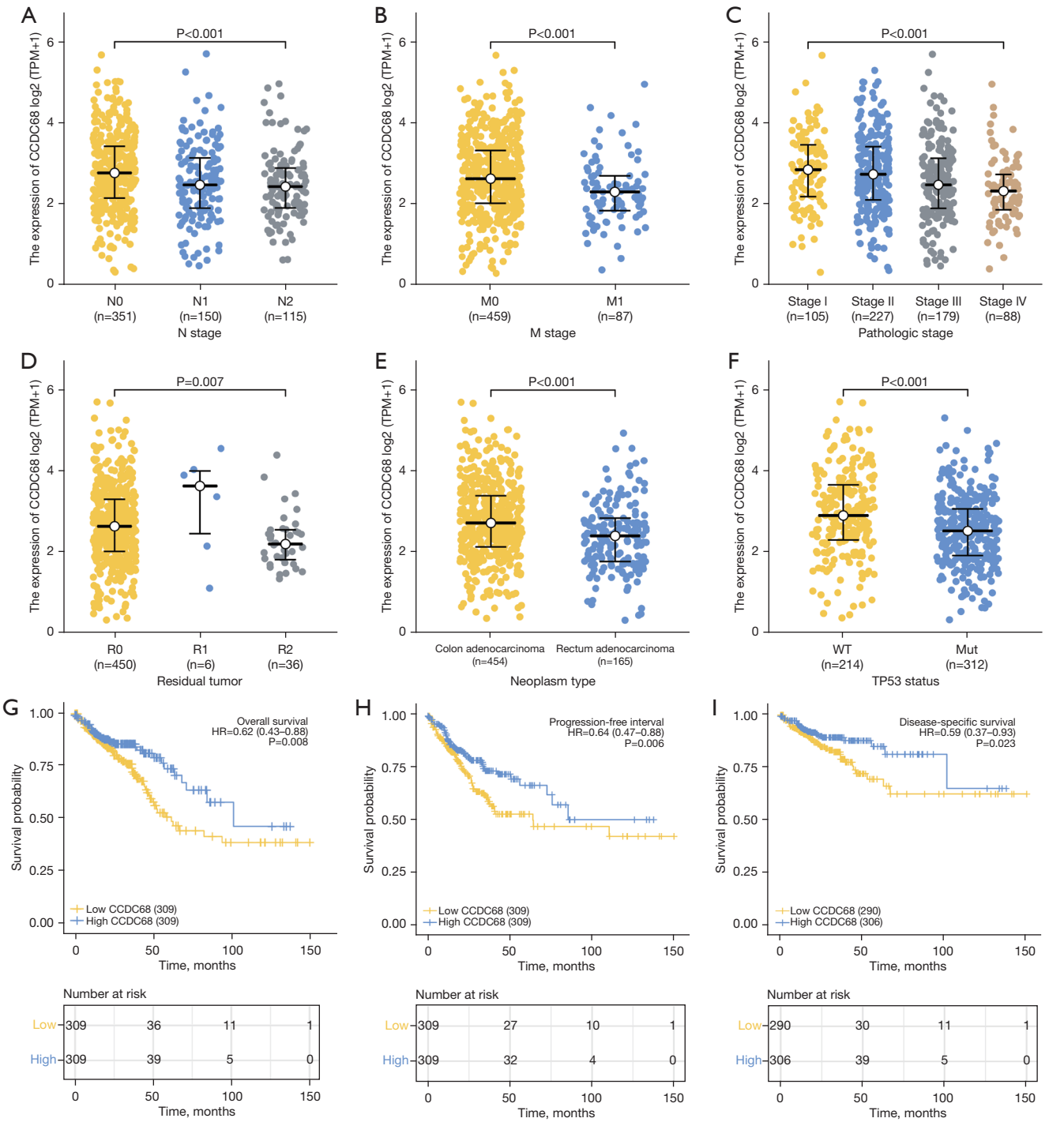


**Figure 2** Association between CCDC68 expression and clinicopathologic characteristics in CRC. (A) N stage, Kruskal–Wallis rank sum test. (B) M stage, Wilcoxon rank sum test. (C) Pathologic stage, Kruskal–Wallis rank sum test. (D) Residual tumor, Kruskal–Wallis rank sum test. (E) Neoplasm type, Wilcoxon rank sum test. (F) TP53 status, Wilcoxon rank sum test. Impact of CCDC68 expression on OS (G), PFI (H) and DSS (I) in patients with CRC in TCGA cohort. CCDC68, coiled-coil domain containing 68; CRC, colorectal cancer; N, lymph node metastasis; M, distant metastasis; OS, overall survival; PFI, progression-free interval; DSS, disease-specific survival; TCGA, the cancer genome atlas.

**Table 1** Association between CCDC68 expression and clinicopathologic characteristics in CRC

| Characteristics | Level | CCDC68 expression | | P value[a] |
|---|---|---|---|---|
| | | Low (n=310) | High (n=309) | |
| T stage, n (%) | T1 | 10 (3.2) | 10 (3.2) | 0.518 |
| | T2 | 46 (14.9) | 59 (19.2) | |
| | T3 | 219 (70.9) | 203 (65.9) | |
| | T4 | 34 (11.0) | 36 (11.7) | |
| N stage, n (%) | N0 | 150 (48.7) | 201 (65.3) | <0.001* |
| | N1 | 88 (28.6) | 62 (20.1) | |
| | N2 | 70 (22.7) | 45 (14.6) | |
| M stage, n (%) | M0 | 218 (77.6) | 241 (90.9) | <0.001* |
| | M1 | 63 (22.4) | 24 (9.1) | |
| Pathologic stage, n (%) | Stage I | 43 (14.3) | 62 (20.8) | <0.001* |
| | Stage II | 99 (32.9) | 128 (43.0) | |
| | Stage III | 97 (32.2) | 82 (27.5) | |
| | Stage IV | 62 (20.6) | 26 (8.7) | |
| Gender, n (%) | Female | 147 (47.4) | 142 (46.0) | 0.776 |
| | Male | 163 (52.6) | 167 (54.0) | |
| CEA level, n (%) | ≤5 | 115 (59.9) | 137 (66.8) | 0.184 |
| | >5 | 77 (40.1) | 68 (33.2) | |
| History of colon polyps, n (%) | NO | 192 (71.1) | 172 (65.6) | 0.207 |
| | YES | 78 (28.9) | 90 (34.4) | |
| Colon polyps present, n (%) | NO | 95 (69.3) | 112 (69.6) | 1.000 |
| | YES | 42 (30.7) | 49 (30.4) | |
| Neoplasm type (%) | Colon adenocarcinoma | 209 (67.4) | 245 (79.3) | 0.001* |
| | Rectum adenocarcinoma | 101 (32.6) | 64 (20.7) | |
| Residual tumor, n (%) | R0 | 214 (87.3) | 236 (95.5) | 0.001* |
| | R1 | 2 (0.8) | 4 (1.6) | |
| | R2 | 29 (11.8) | 7 (2.8) | |
| TP53 status, n (%) | Mut | 170 (66.9) | 142 (52.2) | 0.001* |
| | WT | 84 (33.1) | 130 (47.8) | |
| KRAS status, n (%) | Mut | 109 (42.9) | 105 (38.6) | 0.359 |
| | WT | 145 (57.1) | 167 (61.4) | |
| PIK3CA status, n (%) | Mut | 54 (21.3) | 79 (29.0) | 0.045*,[b] |
| | WT | 200 (78.7) | 193 (71.0) | |
| Age [median (IQR)] | | 68.00 [59.00, 75.75] | 67.00 [58.00, 77.00] | 0.458[c] |
| Height, cm [median (IQR)] | | 170.00 [162.55, 175.00] | 169.00 [162.00, 177.80] | 0.644[c] |
| Weight, kg [median (IQR)] | | 78.00 [65.00, 90.17] | 80.00 [65.20, 93.85] | 0.361[c] |

*, statistically significant. [a], calculated using the Chi-square test. [b], calculated using the Fisher's exact test. [c], nonnormal distribution, calculated using Wilcoxon rank sum test. CCDC68, coiled-coil domain containing 68; CRC, colorectal cancer; CEA, carcinoembryonic antigen; TP53, tumor protein 53; KRAS, kirsten rat sarcoma viral oncogene; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; Mut, mutant; WT, wild type.

 

**Table 2** CCDC68 expression associated with clinical pathological characteristics (logistic regression) in CRC

| Characteristics | Total (N) | Odds ratio in CCDC68 expression | P value |
|---|---|---|---|
| T stage (T3/T4 *vs.* T1/T2) | 617 | 0.98 (0.95–1.01) | 0.123 |
| N stage (N1/N2 *vs.* N0) | 616 | 0.95 (0.92–0.98) | 0.001* |
| M stage (M1 *vs.* M0) | 546 | 0.91 (0.86–0.97) | 0.003* |
| Pathologic stage (Stage III/Stage IV *vs.* Stage I/Stage II) | 599 | 0.95 (0.93–0.98) | 0.002* |
| Neoplasm type (Rectum adenocarcinoma *vs.* Colon adenocarcinoma) | 619 | 0.92 (0.88–0.95) | <0.001* |
| Residual tumor (R1/R2 *vs.* R0) | 492 | 0.95 (0.87–1.01) | 0.127 |
| CEA level (>5 *vs.* ≤5) | 397 | 0.97 (0.93–1.00) | 0.088 |
| TP53 status (Mut *vs.* WT) | 526 | 0.93 (0.89–0.95) | <0.001* |
| KRAS status (Mut *vs.* WT) | 526 | 0.97 (0.94–1.00) | 0.048* |
| PIK3CA status (Mut *vs.* WT) | 526 | 1.01 (0.99–1.04) | 0.311 |

*, statistically significant. CCDC68, coiled-coil domain containing 68; CRC, colorectal cancer; CEA, carcinoembryonic antigen; TP53, tumor protein 53; KRAS, kirsten rat sarcoma viral oncogene; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; Mut, mutant; WT, wild type.

c2.cp.v7.0.symbols.gmt [Curated] was selected as the reference gene set. FDR <0.25 and P.adj <0.05 were considered to be significantly enriched. There were 143 data sets satisfying FDR <0.25 and P.adj <0.05. GSEA showed that the high expression phenotype of CCDC68 was composed of multiple key pathways and biological processes related to tumorigenesis, including the chemokine signaling pathway (NES =1.767, P.adj =0.041, FDR =0.033), the JAK-STAT signaling pathway (NES =1.846, P.adj =0.041, FDR =0.033), FcεRI-mediated NF-κB activation (NES =2.208, P.adj =0.041, FDR =0.033), cell adhesion molecules (CAMs) (NES =1.758, P.adj =0.041, FDR =0.033), complement cascade (NES =1.948, P.adj =0.041, FDR =0.033), FcεRI-mediated MAPK activation (NES =2.506, P.adj =0.041, FDR =0.033), intestinal immune network for IgA production (NES =2.513, P.adj =0.041, FDR =0.033), and the Toll-like receptor signaling pathway (NES =1.777, P.adj =0.045, FDR =0.037) (*Figure 3A-3H*). The Wnt signaling pathway (NES =−2.008, P.adj =0.045, FDR =0.037) in the low CCDC68 expression phenotype was also identified (*Figure 3I*). These results suggest that CCDC68 may promote disease progression by participating in several cancer-related signaling pathways in CRC patients.

### *Correlation between CCDC68 expression and immune infiltration*

Spearman correlation was employed to identify the correlation between CCDC68 and 24 immune cells infiltration quantified by ssGSEA in the CRC tumor microenvironment (*Figure 4A*). CCDC68 expression was significantly linearly correlated with the infiltration level of T helper 2 (Th2) cells (R=0.525, P<0.001) and T helper cells (R=0.408, P<0.001) (*Figure 4B,4C*). The Wilcoxon rank sum test also showed that the infiltration levels of Th2 cells and T helper cells were significantly higher in the high CCDC68 expression samples compared with low expression ones (P<0.001) (*Figure 4D,4E*).

## Discussion

At present, there are only a small number of previous studies on the expression and role of CCDC68 in disease. A combined analysis found genome-wide significant associations of schizophrenia with eight single nucleotide polymorphisms (SNPs) including RS12966547 (CCDC68) (21,22). The homozygous variant in CCDC68 was proposed as a candidate gene for sterility (23). CCDC68 was also expressed in patients with CTCL (9), renal cell (10), CRC (11), and pancreatic ductal adenocarcinoma (12). Although the expression and function of CCDC68 in tumors have been reported, the literature was lacking, and previous studies on CCDC68 were mainly descriptive. To our knowledge, the potential prognostic effect of CCDC68 on CRC has not yet been explored. Therefore, the expression of CCDC68 and its potential role in CRC was

 

**Table 3** Univariate and multivariate cox regression analyzes of prognostic factors for OS, PFI and DSS in CRC

| Characteristics | OS | | PFI | | DSS | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | P value | HR (95% CI) | P value | HR (95% CI) | P value |
| Associations with OS, PFI, DSS and clinicopathologic characteristics in TCGA patients using Cox regression | | | | | | |
| T stage (T3/T4 *vs.* T1/T2) | 2.406 (1.294–4.475) | 0.006* | 3.610 (1.956–6.664) | <0.001* | 6.349 (2.001–20.151) | 0.002* |
| N stage (N1/N2 *vs.* N0) | 2.567 (1.787–3.686) | <0.001* | 2.591 (1.880–3.570) | <0.001* | 4.065 (2.463–6.710) | <0.001* |
| M stage (M1 *vs.* M0) | 4.096 (2.752–6.096) | <0.001* | 5.429 (3.818–7.720) | <0.001* | 7.531 (4.683–12.111) | <0.001* |
| Pathologic stage (Stage III/Stage IV *vs.* Stage I/Stage II) | 2.916 (1.991–4.272) | <0.001* | 2.899 (2.081–4.038) | <0.001* | 5.629 (3.190–9.933) | <0.001* |
| CEA level (>5 *vs.* ≤5) | 2.697 (1.646–4.418) | <0.001* | 2.528 (1.702–3.756) | <0.001* | 2.889 (1.610–5.187) | <0.001* |
| Neoplasm type (Rectum adenocarcinoma *vs.* Colon adenocarcinoma) | 0.742 (0.479–1.151) | 0.183 | 0.879 (0.608–1.270) | 0.491 | 0.645 (0.361–1.151) | 0.138 |
| Age (>65 *vs.* ≤65) | 2.023 (1.371–2.986) | <0.001* | 0.999 (0.729–1.369) | 0.996 | 1.468 (0.924–2.332) | 0.104 |
| Weight (>90 *vs.* ≤90 kg) | 0.756 (0.412–1.388) | 0.367 | 0.999 (0.620–1.610) | 0.998 | 1.067 (0.492–2.314) | 0.870 |
| Height (≥170 *vs.* <170 cm) | 0.773 (0.466–1.282) | 0.318 | 1.376 (0.879–2.154) | 0.162 | 0.821 (0.388–1.734) | 0.604 |
| Gender (Male *vs.* Female) | 1.056 (0.744–1.498) | 0.762 | 1.263 (0.919–1.736) | 0.149 | 1.206 (0.768–1.893) | 0.415 |
| Race (White *vs.* Asian/Black or African American) | 0.933 (0.541–1.610) | 0.803 | 0.619 (0.395–0.970) | 0.036* | 0.493 (0.254–0.959) | 0.037* |
| History of colon polyps (YES *vs.* NO) | 0.832 (0.522–1.326) | 0.440 | 0.827 (0.557–1.228) | 0.346 | 0.988 (0.574–1.698) | 0.964 |
| Colon polyps present (YES *vs.* NO) | 1.316 (0.777–2.229) | 0.307 | 1.070 (0.676–1.693) | 0.774 | 1.386 (0.683–2.811) | 0.365 |
| Residual tumor (R1/R2 *vs.* R0) | 4.466 (2.715–7.347) | <0.001* | 4.062 (2.609–6.324) | <0.001* | 6.140 (3.607–10.453) | <0.001* |
| TP53 status (Mut *vs.* WT) | 1.119 (0.768–1.632) | 0.558 | 1.120 (0.796–1.578) | 0.515 | 0.967 (0.595–1.571) | 0.891 |
| KRAS status (Mut *vs.* WT) | 0.954 (0.657–1.385) | 0.805 | 1.440 (1.031–2.012) | 0.033* | 1.196 (0.739–1.935) | 0.466 |
| PIK3CA status (Mut *vs.* WT) | 0.892 (0.579–1.375) | 0.605 | 0.889 (0.601–1.315) | 0.555 | 0.928 (0.529–1.629) | 0.795 |
| CCDC68 (High *vs.* Low) | 0.615 (0.430–0.880) | 0.008* | 0.641 (0.467–0.880) | 0.006* | 0.589 (0.374–0.928) | 0.023* |
| Multivariate survival model after variable selection | | | | | | |
| T stage (T3/T4 *vs.* T1/T2) | 3.102 (0.719–13.383) | 0.129 | 4.098 (0.532–31.574) | 0.176 | 715034.130 (0.000–Inf) | 0.997 |
| N stage (N1/N2 *vs.* N0) | 0.294 (0.081–1.063) | 0.062 | 0.130 (0.010–1.680) | 0.118 | 0.144 (0.027–0.761) | 0.022* |
| M stage (M1 *vs.* M0) | 1.753 (0.673–4.564) | 0.250 | 6.503 (2.281–18.537) | <0.001* | 13.779 (2.929–64.823) | <0.001* |
| Pathologic stage (Stage III/ Stage IV *vs.* Stage I/Stage II) | 6.073 (1.335–27.621) | 0.020* | 4.601 (0.297–71.155) | 0.275 | 55469487.572 (0.000–Inf) | 0.992 |
| CEA level (>5 *vs.* ≤5) | 1.522 (0.760–3.048) | 0.236 | 2.169 (1.002–4.698) | 0.050* | 1.292 (0.302–5.536) | 0.730 |
| Residual tumor (R1/R2 *vs.* R0) | 1.657 (0.712–3.855) | 0.241 | 17.223 (4.021–73.764) | <0.001* | 17.084 (3.225–90.486) | <0.001* |
| KRAS status (Mut *vs.* WT) | | | 1.621 (0.756–3.473) | 0.214 | | |
| CCDC68 (High *vs.* Low) | 0.498 (0.251–0.990) | 0.047* | 0.457 (0.210–0.994) | 0.048* | 0.188 (0.039–0.909) | 0.038* |

*, statistically significant. OS, overall survival; PFI, progression-free interval; DSS, disease-specific survival; CRC, colorectal cancer; HR, hazard ratio; CI, confidence interval; CEA, carcinoembryonic antigen; TP53, tumor protein 53; KRAS, kirsten rat sarcoma viral oncogene; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; Mut, mutant; WT, wild type; CCDC68, coiled-coil domain containing 68.

 

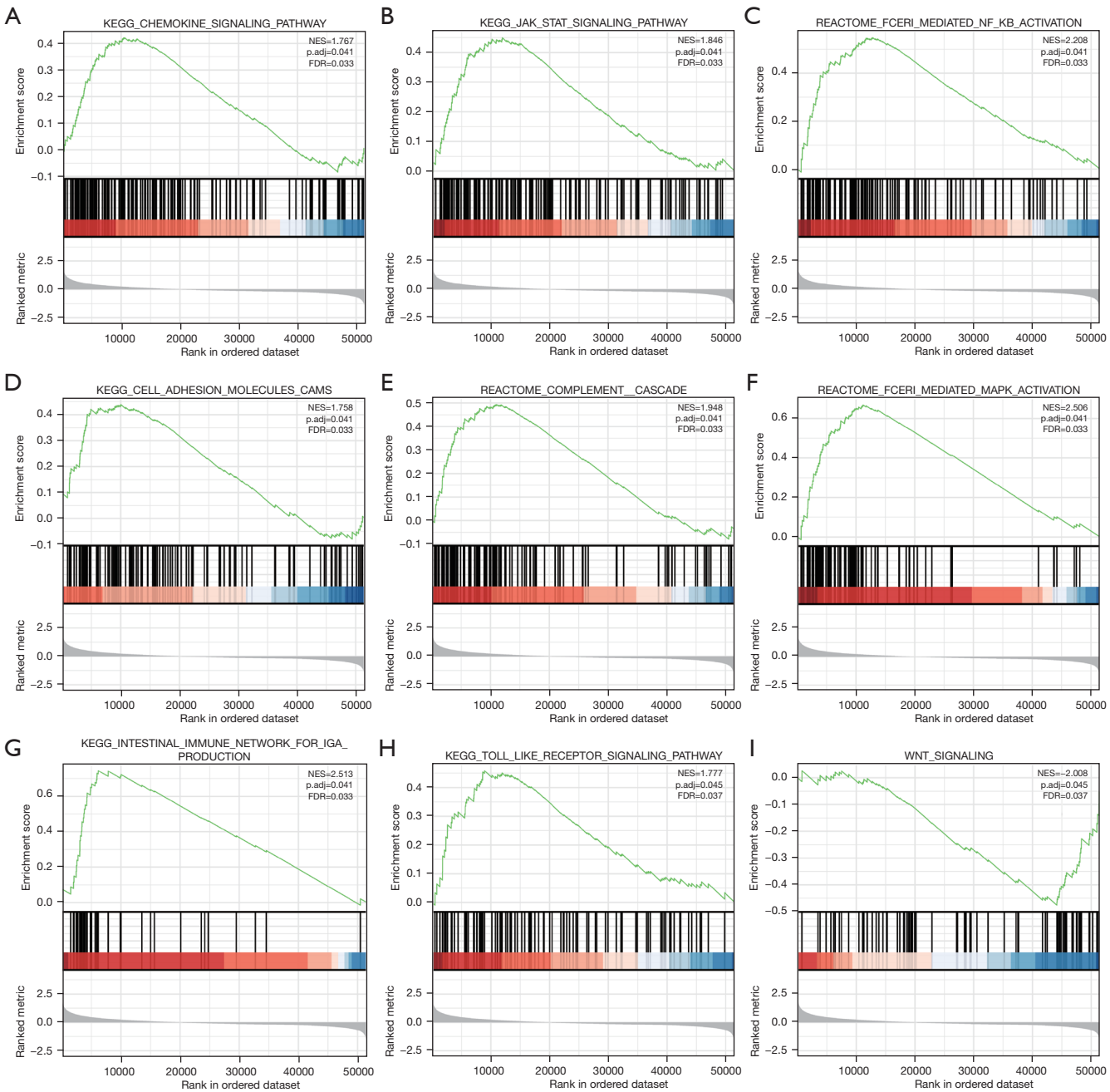


**Figure 3** Enrichment plots from GSEA. Several pathways and biological processes were differentially enriched in CCDC68-related colorectal cancer, including the chemokine signaling pathway (A), the JAK-STAT signaling pathway (B), FcεRI-mediated NF-κB activation (C), CAMs (D), complement cascade (E), FcεRI-mediated MAPK activation (F), intestinal immune network for IgA production (G), the Toll-like receptor signaling pathway (H), and Wnt signaling (I). GSEA, gene set enrichment analysis; CCDC68, coiled-coil domain containing 68; NES, normalized enrichment score; FDR, false discovery rate; JAK-STAT, Janus kinase-signal transducers and activators of transcription; FcεRI, high-affinity IgE receptor; NF-κB, nuclear factor-κB; CAMs, cell adhesion molecules; MAPK, mitogen-activated protein kinase; IgA, immunoglobulin A.

 

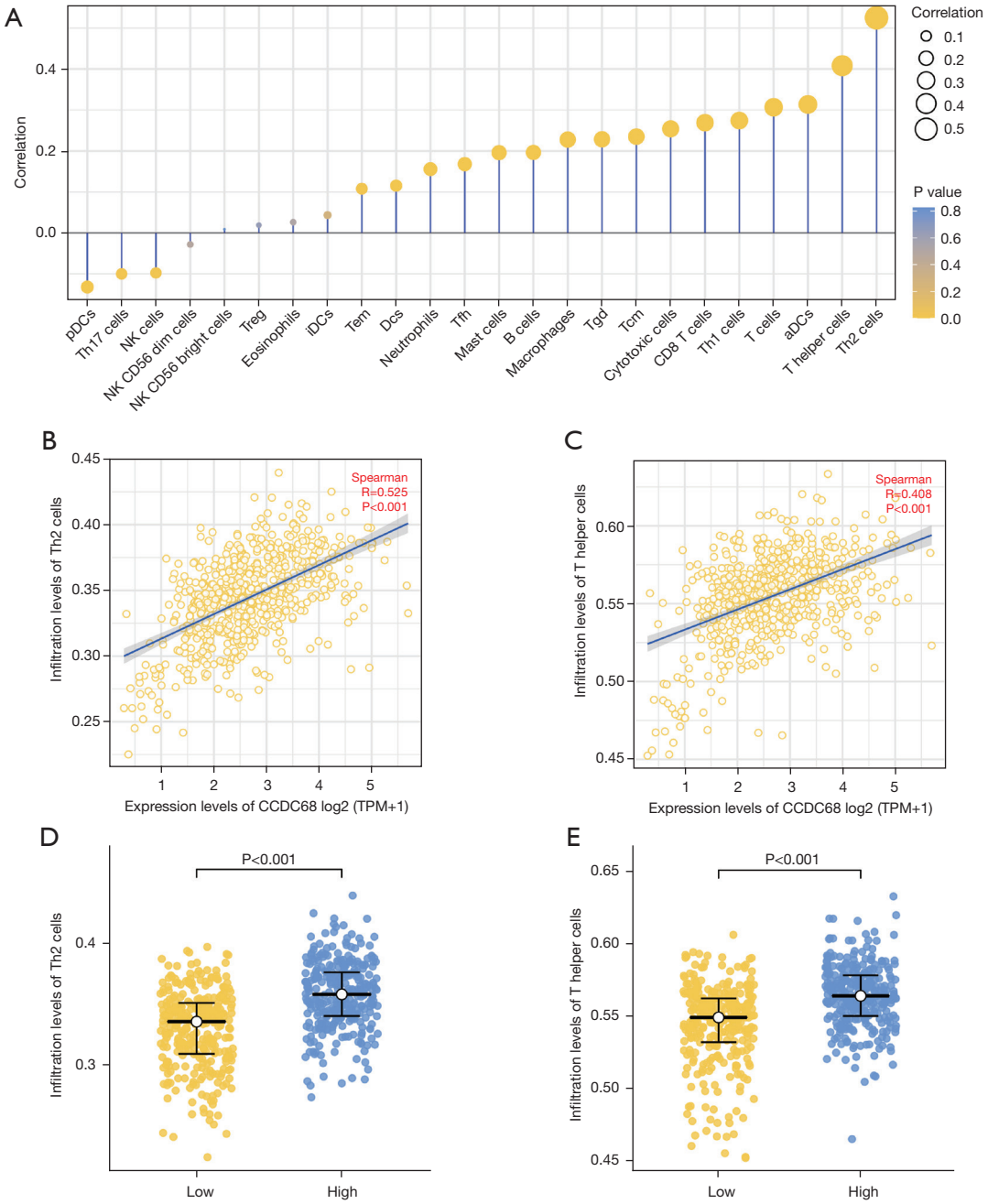


**Figure 4** Association between CCDC68 expression and immune infiltration in the CRC tumor microenvironment. (A) Correlation between the relative abundances of 24 immune cells and CCDC68 expression levels. The size of dots shows the absolute value of Spearman R. (B,C) Correlation between CCDC68 expression levels and the infiltration levels of Th2 cells (B), T helper cells (C), Spearman correlation. (D,E) Correlation between high- and low-CCDC68 expression and the infiltration levels of Th2 cells (D), T helper cells (E), Wilcoxon rank sum test. CCDC68, coiled-coil domain containing 68; CRC, colorectal cancer; DCs, dendritic cells; aDCs, activated DCs; iDCs, immature DCs; pDCs, plasmacytoid DCs; Th, helper T cells; Tcm, T central memory; Tgd, T gamma delta; Tfh, T follicular helper; Tem, T effector memory; Treg, regulatory T cells.

 

the focus of the present study.

In this study, bioinformatics analysis using TCGA high-throughput RNA sequencing data demonstrated that a reduced expression of CCDC68 in CRC was associated with advanced clinical pathologic characteristics (lymph node metastasis, distant metastasis, pathologic stage, residual tumor, neoplasm type, TP53 status), survival time, and poor prognosis. In addition, we used GSEA and ssGSEA to investigate the role of CCDC68 in CRC. GSEA showed that the chemokine signaling pathway, the JAK-STAT signaling pathway, FcεRI-mediated NF-κB activation, CAMs, complement cascade, FcεRI-mediated MAPK activation, intestinal immune network for IgA production, and the Toll-like receptor signaling pathway were differentially enriched in the high CCDC68 expression phenotype, while the Wnt signaling pathway was significantly enriched in the low CCDC68 expression phenotype. SsGSEA showed that CCDC68 expression was positively correlated with Th2 cells and T helper cells. These results indicated that CCDC68 might act as a potential prognostic marker and therapeutic target in CRC.

CCDC68 is a protein coding gene, and the centriolar protein is required for centriole subdistal appendage assembly and microtubule anchoring in interphase cells (24). The microtubule plays a crucial role in cell proliferation, differentiation, migration, and tumor occurrence. Increased microtubule assembly rates mediated chromosomal instability in CRC cells (25). A recent study showed that metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) sponges microRNA (miR)-106b-5p to promote the invasion and metastasis of CRC via SLAIN motif family member 2 (SLAIN2) enhanced microtubules mobility (26). In this work, we demonstrated that CCDC68 was significantly down-regulated in CRC compared with normal or adjacent normal tissues, which was consistent with the findings of Sheffer M (15). Meanwhile, reduced expression of CCDC68 in CRC was associated with advanced clinical pathologic characteristics and predicts poor prognosis. A similar result was observed in a pancreatic ductal adenocarcinoma experiment, which showed that CCDC68 had a negative impact on tumor biology, and this was the first study to elucidate the anticancer effect of CCDC68 in cancer (12). However, the expression of CCDC68 in pancreatic adenocarcinoma (PAAD) was significantly up-regulated compared with the normal sample (*Figure 1D*). These results seem to be contradictory and need to be verified by more experiments in the future.

Due to the limited data on CCDC68 function, we performed functional annotation based on GSEA, and demonstrated that CCDC68 was involved in the chemokine signaling pathway, the JAK-STAT signaling pathway, FcεRI-mediated NF-κB activation, CAMs, complement cascade, FcεRI-mediated MAPK activation, intestinal immune network for IgA production, the Toll-like receptor signaling pathway, and the Wnt signaling pathway. Recent research has shown that chemokine-mediated chronic inflammation was a direct cause of colitis-associated cancer (CAC) (27). The JAK/STAT signal may be used as a novel tumor marker and prognostic factor for the diagnosis, assessment, and prognosis of colon cancer (28). The NF-κB signaling pathway has been shown to be a key regulator of inflammation and is important in the carcinogenic process of CRC (29). Tumor cell adhesion is a key step in peritoneal dissemination, and sialyl Lewis a ($sLe^a$) and mucin 16 (MUC16) were the most promising prognostic biomarkers for colorectal peritoneal metastases (30). The complement system is an important component of the inflammatory response, and inflammation is involved in various stages of tumorigenesis and cancer progression (31). The majority of CRCs follow a conventional pathway, which is initiated by activating mutations of the Wnt pathway; however, 10–15% of CRCs are thought to be initiated by activating mutations in the B-Raf proto-oncogene, serine/threonine kinase (BRAF) oncogene, which amplifies MAPK signals and drives the serrated tumor pathway in CRC (32). Activation of the intestinal immune network for the IgA production signaling pathway contributes to cell proliferation and migration of HCC cells (33). Persistently positive toll-like receptors (TLRs) expression and lower expression of TLR inhibitors is associated with higher TLR protein levels throughout the spectrum of lesions of colon carcinogenesis (34). All of the above are conventional pathways of tumorigenesis, indicating that CCDC68 acted on colon cancer in multiple ways. A recent mechanistic study suggested that CCDC68 downregulation promoted CRC growth by inhibiting itchy E3 ubiquitin protein ligase (ITCH/AIP4)-mediated cyclin-dependent kinases 4 (CDK4) degradation (35). However, more data are needed to elucidate the potential regulatory mechanism of CCDC68 in CRC.

In addition, we performed ssGSEA and Spearman correlation to reveal connections between CCDC68 expression and immune infiltration levels in CRC. Our findings showed that CCDC68 expression was significantly positively correlated with Th2 cells and T helper cells. One previous study suggested that patients with high Th1

 

cluster expression had prolonged disease-free survival in CRC, whereas none of the Th2 clusters were predictive of prognosis (36). A recent study obtained similar results; compared with healthy subjects, the number of Th1 cells and the Th1/Th2 ratio of CRC patients were significantly reduced, while the number of Th2 cells was not significantly different (37). However, contrary to our results, Tabata *et al*. (38) demonstrated the Th2 subset dominance in patients with digestive cancers. Summarize our findings, CCDC68 might play an important role in the recruitment and regulation of immune infiltrating cells in CRC. However, the results of Th2 cells were controversial, and will be the entry point for our future research.

Although CCDC68 seemed to be a potential biomarker of prognosis in clinical application, there were some limitations in this study that should be noted. Firstly, the data used in our study were accessed from a public database, so the quality of these data cannot be evaluated. Secondly, the sample size of our clinical validation set was insufficient to cover different races and regions, which might affect CCDC68 expression. Thirdly, due to the absence of experiments, our results cannot be verified. To further investigate the mechanism of CCDC68 in CRC, some wet experiments should be carried out in future research.

In conclusion, the expression of CCDC68 may be a potential prognostic molecular marker for poor survival in CRC. Moreover, CCDC68 may participate in the development of CRC via multiple signaling pathways, such as the chemokine signaling pathway, the JAK-STAT signaling pathway, FcεRI-mediated NF-κB activation, CAMs, complement cascade, FcεRI-mediated MAPK activation, intestinal immune network for IgA production, the Toll-like receptor signaling pathway, and the Wnt signaling pathway. In addition, CCDC68 potentially contributes to the regulation of Th2 cells and T helper cells in CRC. Our results warrant further studies on the mechanism of CCDC68 promotion of CRC tumor progression.

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## Footnote

*Reporting Checklist:* The authors have completed the REMARK reporting checklist Available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-148/rc

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://jgo.amegroups.com/article/view/10.21037/jgo-22-148/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (revised in 2013) and complied with the publication guidelines provided by TCGA, and does not include any research on human participants or animals by any author.



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